REMARKS/ARGUMENTS

Claims 28-29 and 31-54 are pending.

Claims 28 and 42 have been amended.

Claim 30 has been cancelled.

Claims 45 and 47-54 have been withdrawn.

Support for the amendments is found in the claims and specification (page 15), as originally filed. In addition, claim 1 comprises the limitations of claim 30 and claim 42 comprises the limitations of claim 12.

No new matter is believed to have been added.

Applicants wish to thank the Examiner for indicating the allowable subject matter of claims 43-44.

Applicants also thank the Examiner for a discussion on August 13, 2007. The undersigned Applicants' representative explained that the Van Ness et al. method does not involve a trifunctional reagent having an arrangement of the claimed tripod and the intensity of the measured fluorescence is inversely proportional to the quantity of the bound analyte. Further, a combination of the assay of Van Ness et al. and Lee et al. is improper. Also, one would not have been motivated to combine two assays with a reasonable expectation of success because the assays are based on different principles. The rejection over Plowman et al. was also discussed.

Claims 28, 30-42 and 46 are rejected under 35 USC 103(a) over Van Ness *et al.* (US 5,232,830) in view of Lee *et al.* (*J. Agr. Food. Chem.*, 1999, vol 47, p. 2766-2770). The rejection is traversed because the combination of the references does not describe:

1) a trifunctional reagent;

2) regeneration of a solid support;

3) measuring the intensity of a signal emitted by a luminescent group L on a solid support, which is proportional to the amount of an analyte to be detected; and

4) one would not have been motivated to used the Lee *et al.* solution-based fluorescent excitation transfer assay in the Van Ness *et al.* sandwich solid support assay because the Van Ness *et al.* and Lee *et al* assays are based on different principles.

Subject matter and advantages of the present invention

Claim 28 is directed to a method for detection of an analyte in a fluid sample, comprising:

1) saturating a solid support comprising, on at least part of its surface, at least one trifunctional reagent (tripod Y) comprising the following three functional poles:

i) a luminescent group (L),

ii) a molecule (B) chosen from the analyte a, an analog of the analyte a or a fragment of the analyte; and

iii) a function that provides attachment of the trifunctional reagent to the surface of the solid support,

with a receptor for the analyte, the receptor being labeled with a compound (Q) (receptor-Q) that quenches the luminescence of the group L, so as to form a complex C between the molecule (B) and the receptor-Q;

2) bringing the solid support into a contact with a fluid sample that may comprise the analyte to be detected;

3) measuring the intensity of the signal emitted by the group L, which is proportional to the amount of analyte present in the fluid sample; and

4) regenerating the solid support by bringing the solid support into contact with the receptor-Q.

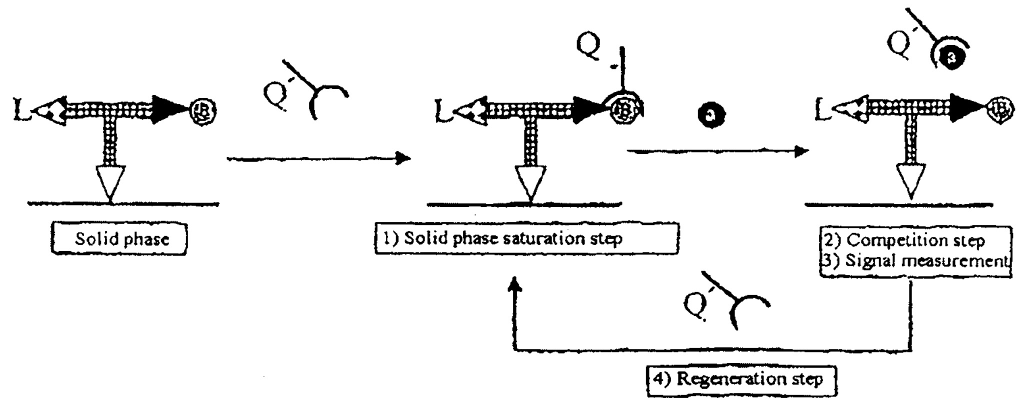
The claimed method is a method for *continuous* (see page 11 of the present specification) *heterogeneous*-phase (see page 2, lines 25-30 of the present specification) detection of analytes in a fluid sample that makes possible to avoid any incubation, prior to the detection step, of the analyte with a labeled antibody or a labeled analyte and other drawbacks of the know methods described in the present specification on pages 1-10.

According to the claimed method:

- step 1) allows complexation of the molecule (B) with the receptor-Q. At the end of this first step, the luminescence L is decreased or suppressed;
- in step 2), bringing the sample into contact with the surface of the solid support will induce, when the sample contains the analyte, competition between the analyte and the molecule (B) and formation of the complex with the receptor-Q. The binding of the analyte to the receptor-Q will result in the receptor-Q being eliminated from the surface of the solid support and in the luminescence emitted by the compound (L) present on the tripod Y being restored;
- the intensity of the signal measured in step 3) is then *proportional* to the amount of analyte present in the sample to be analyzed;
- the regeneration step 4) will again bring about complexation of the receptor-Q on the tripod Y and thus suppression of the luminescence of L, so as to allow further detection of the analyte in a new sample.

The principle of the method for detection of an analyte in accordance with the invention is represented by scheme A below:

SCHEME A



The method of detection in accordance with the invention has the following advantages:

1) due to the specific structural conformation of the tripod Y used during the method, the regeneration of the solid phase can be carried out very readily, without an alteration of its properties. In fact, in the claimed method, the signal is measured after formation of a complex bound to the solid phase. Even though some of known assaying methods allow several successive assays, they are however limited in number and require a solid support regeneration step that is often long and restricting, which results in the dissociation of the complex formed. In addition, the drastic conditions for carrying out the regeneration steps (passing over acid or basic solutions) mean that the detection method must be stopped, thus prohibiting any application of these methods to *continuous assays* of a given analyte.

In the claimed method, the presence of the analyte in the sample brings about dissociation of the receptor-Q and of the molecule (B); *the regeneration step therefore consists quite simply in reforming this complex by adding receptor-Q*. The regeneration does not therefore involve the use of acid or basic solutions that may impair the properties of the molecules of the solid phase or an exchange reaction between two molecules at the receptor binding site, the kinetics of which are longer than the reaction to form a complex;

2) the signal is measured in the region on which the tripod Y was immobilized, which makes it possible to obtain a localized signal, while in the known assays, a signal bound to molecules in solution is measured at the outlet of a capillary;

3) since the signal is localized, several molecules may be detected simultaneously on the same solid support by attaching to distinct and known zones several types of tripods Y that differ from one another through the nature of the molecule (B) that they comprise;

4) the signal measured corresponds to all the molecules of the analyte that have been in contact with the solid support between two regenerations. Thus, the claimed method allows permanent monitoring while at the same time taking measurements that are spaced out over time;

5) this assay format is applicable to all molecules since it does not require the simultaneous binding of two receptors to the analyte, as is necessary according to the assay methods previously known, and which require the analyte to be of sufficient size;

6) the presence of the analyte in the sample results in the appearance of a signal, unlike most of the known competition assays, that allows an easier detection;

7) finally, since the detection system uses the energy transfer phenomenon, it also possible to detect and quantify the presence of an the analyte by means of the variation in luminescence of the compound Q if it is fluorescent or the variation in the apparent time for decrease in luminescence of the compound (L).

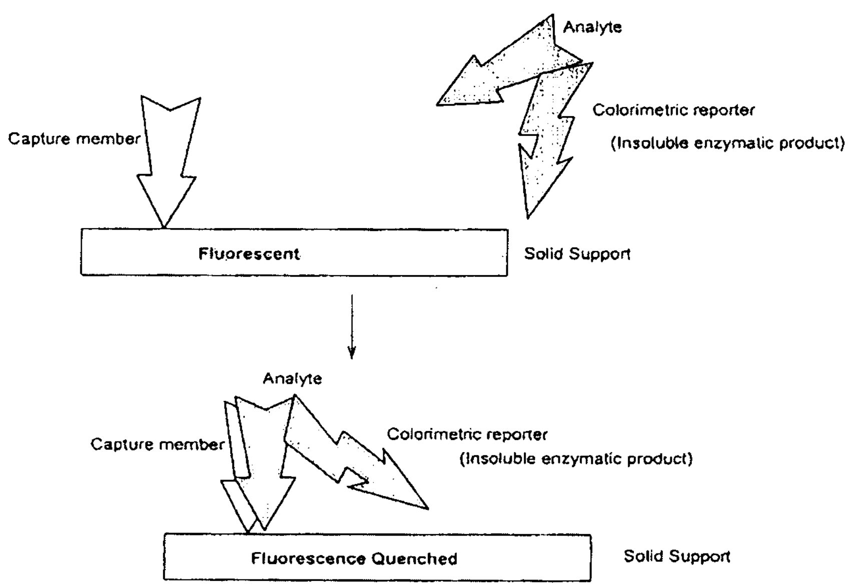
Van Ness et al. describe a method and composition (a sandwich assay) for detecting a member of a ligand pair on solid *supports having intrinsic fluorescence.*

The Van Ness et al. method includes contacting a target member of a ligand pair with a capture member of the ligand pair, wherein the *capture member is immobilized on solid support having intrinsic fluorescence, and the contacted ligand pair is in association with a colorimetric reporter*; irradiating the solid support, wherein the solid support is attached to

the ligand pair in association with the colorimetric reporter; and determining the resultant fluorescence (col. 2, lines 20-31; the Examples).

The intrinsic fluorescence of the support is quenched by the colorimetric reporter (col. 4, lines 14-23) which is defined as being *an insoluble enzymatic product* (col. 2, lines 39-45; col. 4, lines 24-33), *the colorimetric reporter being attached to the target ligand pair* (col. 4, lines 14-23).

This arrangement can be represented by the following schemes:



Even if the capture member is attached to the support *via* a spacer arm bearing an amine function as noticed by the Examiner, *the capture member does not contain a luminescent group.*

Homo- and hetero trifunctional reagents mentioned in Van Ness et al. (col. 5, line 47, to col. 6, line 19) are not equivalent (and do not lead) to the claimed tripod reagent. The Van Ness et al. trifunctional reagents are used for attaching capture oligonucleoitde probes to beads having intrinsic fluorescence coated with a polymer (col. 5, lines 17-22). For example, an amino-tailed oligonucleotide can be activated with a mono (or multi)-functional reagent

(e.g., cyanuric chloride) wherein an alkylamino dichlorotriazine is formed, which is then reacts toward the amine-contained polymer (col. 5-6, the bridging paragraph; col. 10, lines 1-20; Example 1).

Therefore, contrary to the Examiner's believe, the Van Ness et al. method *does not involve a trifunctional reagent* having an arrangement of the claimed tripod and *the capture member does not contain a luminescent group.*

On the contrary, the colorimetric reporter is "in association" with the target member (analyte) (col. 4, lines 14-23).

Once the immobilized ligand pair is formed, the solid support is irradiated (col. 4, lines 34-45; the Examples). If the colorimetric reporter is an enzyme, a substrate appropriate to that enzyme is added, and the substrate is converted to enzymatic products on the solid support (that will quench the fluorescence of the support), and then the solid support is irradiated.

The intensity of the measured fluorescence is *inversely proportional* to the quantity of preferred enzymatic product deposited on the solid support (which is the contrary according to the present invention), and the quantity of enzymatic product is directly proportional to the quantity of the bound analyte (col. 4, lines 46-58). Because the quantity of the preferred enzymatic product produced is proportional to the quantity of the bound analyte and the quenching of fluorescence by the colored enzymatic product is proportional to the quantity of the produced product, the method allows quantitative determination of the bound analyte.

Thus, the principle of the method disclosed in Van Ness *et al.* is totally different from that of the method of claim 28.

Further, Van Ness *et al.* implies a preliminary modification of the analyte by a colorimetric reporter which is one of the drawbacks the present invention allows to avoid (see page 10, lines 3-8 of the present specification).

In addition, Van Ness *et al.* describe that the support must be separated from the fluorescent product to allow the quantification of fluorescence:

> However, the fluorescent product must be measured in 411 environment that neither quenches the product nor is fluorescent itself. Because the solid supports described herein possess a very high intrinsic fluorescence in their native state, it is act possible to accurately measure a fluorescent product in the presence of these solid supports. The solid support must therefore be removed from the substrate solution or the solution decanted and placed in a separate vesicle to allow accurate determination of fluorescence. (Col. 6, line 65 to col. 7, line 6)

In the claimed method, fluorescence is detected without dissociating the tripod comprising luminescence from the solid support.

Thus, Van Ness *et al.* teach away from the claimed method.

In addition, the Van Ness *et al.* method is *not* continuous and requires special regeneration procedures and using new reagents before the next round of measurements is performed (see the Examples) (see the desecration of known methods in the present specification, pages 1-10).

Also, one would not have been motivated to used the Lee *et al.* solution-based fluorescent excitation transfer assay in the Van Ness *et al.* sandwich solid support assay possessing intrinsic fluorescence with a reasonable expectation of success because the Van Ness *et al.* and Lee *et al* assays are based on different principles, which when combined, yield an unworkable model:

1) fluorescent quenching in Van Ness *et al.* employs a *solid support possessing an intrinsic fluorescence* that fluoresces when irradiated with UV light (col. 2, lines 36-52). A colorimetric reporter (insoluble enzymatic products) deposited on the solid support via a target ligand pair quenches the fluorescence of the *solid support* and yields a means to quantify the product (col. 2, lines 36-52; the Examples). Lee *et al.* solution-based fluorescent excitation transfer assay uses a competition between a labeled and unlabeled spinosyne

(analyte) for binding to a quencher-labeled antibody wherein the decrease of quenching is caused by dissociation of the complex between a donor-labeled spinosyn (F-Ag) and the quencher-labeled antibody (TMR-Ab);

2) in the Van Ness *et al.* assay, the intensity of the measured fluorescence is *inversely proportional* to the quantity of preferred enzymatic product deposited on the solid support (which is the contrary according to the present invention), and the quantity of enzymatic product is directly proportional to the quantity of the bound analyte (col. 4, lines 46-58). Because the quantity of the preferred enzymatic product produced is proportional to the quantity of the bound analyte and the quenching of fluorescence by the colored enzymatic product is proportional to the quantity of the produced product, the method allows quantitative determination of the bound analyte. In the Lee *et al.* assay, a donor-labeled spinosyn (F-Ag) is bound to a quencher-labled antibody (TMR-Ab) (page 2768, left col.). The addition of non-labled Ag to the solution reduced the fluorescence quenching (page 2768, right col.), i.e., the dependency of fluorescence v. analyte is proportional; and

3) the Lee *et al.* assay is homogeneous (i.e., the assay carried out in a liquid medium. flow in solution), while the Van Ness *et al.* describe a heterogeneous assay (i.e., detection of analytes in a fluid sample carried out on a solid support).

Thus, combining the Van Ness *et al.* assay based on quenching fluorescence of a solid support by a colorimetric reported associated with a target ligand pair with the Lee *et al.* assay based on quenching a donor-labeled antigen by a quencher-labeled antibody in solution is improper. Also, the Van Ness *et al.* assay the Lee *et al.* assay are based on different principles of measuring fluorescence and are solid verses liquid assays so that combining the two assays does not produce a workable model.

Also, Lee *et al.* do not cure the deficiency of the Van Ness *et al.* method. Even if Lee *et al.* disclose a method according to which the fluorescence of a receptor is quenched by a

reporter, neither Van Ness *et al.* nor Lee *et al.* disclose a trifunctional reagent and a regeneration of a solid support as claimed.

The Examiner believes that Lee *et al.* describe regeneration of a solid support (see page 8, paragraph 40 of the Official Action). Applicants respectfully disagree. Lee *et al.* describe in "Conclusion" that "[w]e observed fluorescence quenching of F-spinosyn after mixing with TMR-Ab and restoration of fluorescence after adding excess spinosyn A to the reaction solution" (page 2769, right col.). Thus, Lee *et al.* do not describe a solid support assay and restoration of the solid support. Lee et al. describe that when a donor-labeled spinosyn (F-Ag) is bound to a quencher-labled antibody (TMR-Ab), fluorescence is quenched. To "free" (i.e., restore) fluorescence that allows detecting an analyte, an unlabeled spinosyn is added (an analyte) which competes for binding to the quencher-labeled antibody. Since the quencher is released from the complex with the labeled spinosyn during the competition, quenching is decreased and fluorescence is "restored".

Therefore, substituting the compounds Lee *et al.* into the Van Ness *et al.* assay still does not produce the claimed method.

Applicants request that the rejection be withdrawn.

Claims 29 is rejected under 35 USC 103(a) over Van Ness *et al.*, Lee *et al.*, and Plowman *et al.*, Anal. Chem., 71:4344-52 (1999). The rejection is traversed because the combination of the references does not describe:

(1) a trifunctional reagent;

(2) regeneration of a solid support;

(3) measuring the intensity of a signal emitted by a luminescent group L on a solid support, which is proportional to the amount of an analyte to be detected; and

(4) one would not have been motivated to used (a) the Lee *et al.* solution-based fluorescent excitation transfer assay and (b) an integrated optical waveguide used to evanescently excite fluorescence from a multianalyte sensor surface in a sandwich assay; multiple channels; pre-mixed analytes and labeled tracer antibodies of Plowman *et al.,.* in the Van Ness *et al.* sandwich solid support enzyme-based assay because the Van Ness *et al,* Plowman *et al.,* and Lee *et al.* assays are based on different principles.

The disclosure of Van Ness *et al.* and Lee *et al.* is described above.

Plowman *et al.* do not cure the deficiency. Plowman *et al.* describe a sandwich immunoassay for detecting multiple analytes (fig. 1) in which a capture antibody is attached to a solid support and a *pre-mixed* analyte with a tracer antibody labeled with a dye is passed over the capture antibody (fig. 1; Material and Methods, pages 4346-47).

Plowman *et al.* do not describe tripoids as claimed and regeneration of a solid support ("regeneration of the surface was not considered," page 4347, right col., line 10). Thus, substituting the Plowman *et al.* assay into that of Lee *et al.* and Van Ness *et al.* assay still does not produce the claimed method.

In addition, Plowman *et al.* describe an assay based on a completely different principle (e.g., integrated optical waveguide used to evanescently excite fluorescence from a multianalyte sensor surface in a sandwich assay; multiple channels; pre-mixed analytes and labeled tracer antibodies) and uses a different set up and reagents compared to Van Ness *et al.* and Lee *et al.* so that detecting multiple analytes in the Van Ness *et al.* and Lee *et al.* assay by using the principle of Plowman *et al.* is not possible.

Thus, Van Ness *et al.*, Lee *et al.*, and Plowman *et al.* do not make claim 29 obvious. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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